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VOLATILE FLAVOR COMPOUNDS COMPOSITION OF FRESH AND STEAMED TIGER GROUPER FISH (*Epinephelus fuscoguttatus*)

Rusky Intan Pratama, Lena Lutfina Natakusumawati, Juniato and Walim Lili

Laboratory of Fisheries Processing Product, Padjadjaran University, Indonesia. E-mail : lenalutfina16@gmail.com

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ABSTRACT

The objectives of this study was to identify fresh and steamed tiger grouper fish (*Epinephelus fuscoguttatus*) volatile flavor compounds. Grouper fish was taken from Indramayu West Java, then sample preparation was conducted at The Laboratory of Fishery Processing Technology, Faculty of Marine Science, University of Padjadjaran. Volatile components were analyzed at Flavor Laboratory, Indonesian Centre for Rice Research, Sukamandi, Subang in February to April 2018. The methods used in this study was to detected volatile compounds using Gas Chromatography/ Mass Spectometry (GC/MS) with sample extraction temperature were 40˚C for fresh and 80˚C for steamed grouper fish. The proximate analysis was also done on those two samples. The volatile compound analysis successfully detected 11 compounds in fresh grouper fish and 19 volatile compounds were detected in steamed grouper fish. These could occurred as a result of the forming of more volatile compounds in account of heating process, thermal oxidation and fatty acids decomposition. The volatile compounds that were detected came from hydrocarbons, aldehydes, alcohols, ketones groups. The proximate analysis showed differences between two treatments in fresh grouper fish had 77,77% water content, 1,97% ash, 18,21% protein, 0,66% fat and steamed grouper fish had 70,76% water content, 2,04% ash, 25,56% protein, 1,64% fat.

INTRODUCTION

Indonesia is a maritime country with an abundant of fisheries resources that are very abundant because two-thirds of Indonesia's territory consists of the sea. Indonesia's fishery resources have excellent potential to contribute to the nutritional fulfillment of Indonesians and in support of national food security (Rini 2016). Fish is as an alternative fulfillment of nutritional needs for the community, along with the increasingly expensive sources of protein such as chicken and beef (Main 2010). Fish is one of the food sources whose nutritional value is very good because it contains protein of 16 - 24 percent of its weight. In addition, fish meat contains fat between 0.2 - 2.2 percent, carbohydrates, minerals and vitamins. Fish meat is also suitable for consumption by patients with high blood pressure because of its low cholesterol content (Susanto 2005).

Tiger grouper (*Epinephelus fuscoguttatus*) is one of the leading fish commodities that are easy to be cultivated in bulk with simple technology and clear market prospect. This fish is of high nutritional value and can be cultivated commercially in several tropical countries. The delicious taste of the meat makes this fish have high value in the world market. Fish grouper fishing activities in Indonesia are increasingly encouraged in line with the increasing demand for grouper, both to fulfill the domestic and export commodities that are increasingly in demand. Indonesia ranks third as a largest producer of grouper fish products by giving around (12.1%) share of total world grouper fish production (FAO 2013).

Food processing by heating can be done in various ways. According to Gardjito (2013) examples of high-temperature processing are steaming (wet heating). Steaming or using steam as a heat source has the advantage that vitamins and other heat-sensitive food components have a smaller risk of loss (Fellows 2000). Steaming as one form of processing using high temperatures is also expected to affect the composition of flavor compounds in fishery products (Pratama et al. 2013).

Flavor is one of the important factors in the acceptance of a food in the community. Flavor generally consists of a volatile flavor derived from a group of alcoholic compounds, ketones, aldehydes, sulfur and nitrogen-containing compounds, hydrocarbons, heterocyclic compounds, and esters (Tanchotikul & Hsieh 1989 in Liu et al 2009). The volatile component is a flavor component that acts as the aroma perceived by the aroma receptors of the olfactory organ such as the olfactory system of the nasal cavity. Flavor molecules released from food into the oral cavity and volatile flavor components move through the nasopharynx in the nose when food is eaten (Pratama 2011). The purpose of this study was to determine any volatile compounds found in fresh and steamed grouper

METHOD OF RESEARCH

Place and Time of Research

The samples were taken from Karangsong fish landing site, Indramayu District, West Java, Indonesia. Samples preparations were carried out at Fisheries Product Processing Laboratory, Fisheries and Marine Sciences Faculty, Padjadjaran University. Volatile compounds analysis were carried out at Flavor Laboratory, Rice Research Centre Office, Sukamandi, Subang and proximate analyses were carried out at Inter-University Centre Laboratory, Bogor Agriculture Institute. The research was conducted in February – April 2018.

Samples Preparation

Grouper samples as much as 5 kg were taken from TPI Karangsong, West Java using a cool box with a basic layer given 5 cm thick ice cubes to prevent the propagation of heat from the air on the outside. The thickness between the layers of fish and ice should be the same and try to keep each fish covered with ice so that it would be cool faster, then at the edge added the ice layer as thick as 7 cm (Suprayitno 2017). Cool box which already contained fish samples was taken to the UNPAD Fisheries Product Processing Laboratory to be prepared.

The fish were divided into two groups of 250 g each. The preparation process for the fresh fish treatment were the fish was washed first and then cut into fillet after that grouper meat was labelled and packaged in three different packaging layers. The first primary packaging was aluminium foil, the secondary packaging was cling wrap plastics and the tertiary packaging is a zip-lock plastic bags. The preparation process for the steamed treatment were the fish was washed first and then steamed using a temperature of 100˚ C after that the meat part was taken, then the fish was labelled and packaged, the packaging method is the same as packaging in a fresh fish sample. Each sample might be marked using label paper. This was done to minimize the changes and damages that occur to the sample flavor to be analyzed. These changes and damages could be caused by air, light, and temperature (Pratama 2011). The finished packaged sample was put into a low temperature cool box to be transported to the laboratory to be tested for volatile compounds and proximate tests

Data Analysis

The volatile compound analysis procedure performed was a modification of the procedure study performed in the Guillen & Errecalde (2002). Analysis of volatile compounds was performed using a series of Gas Chromatography instruments (Agilent Technologies 7890A GC System) and Mass Spectrometry (Agilent Technologies 5975C Inert XL EI CI / MSD). The sample extraction was done by Solid Phase Micro extraction (SPME) method using DVB / Carboxen / Poly Dimethyl Siloxane fiber with 40o C heating temperature for fresh samples and 80o C for steam samples for 45 minutes (in water bath). The GC columns used are HP-INNOWax (30m x 250µm x 0.25µm), helium-carrying gas, 45 (hold 2 min) starting temperature, 6 / min temperature rise, tool end temperature 250 (hold 5 minutes) with 45 minutes. The mass spectra of the detected compound were compared with the mass spectra pattern contained in the NIST data center or library version 0.5a (National Institute of Standard and Technology) on the computer database. The components of the volatile flavor compound were further analyzed using the Automatic Mass Spectral Deconvolution and Identification System (AMDIS) software (Mallard and Reed 1997). Proximate analyzes conducted on fresh and steamed grouper samples include moisture, ash, protein and fat content based on AOAC procedure (2005).

The results of the analysis of the resulting volatile compounds would be discussed in a comparative descriptive manner based on semi-quantitative identification and intensity of the detected compounds in the tested sample (Pratama 2011). The data obtained from the proximate test of all samples were calculated the average value and standard deviation based on Steel and Torrie (1983), then discussed descriptively

RESULT AND DISCUSSION

Analysis of Volatile Compounds

The results of the analysis of volatile flavor compound of tiger grouper (*Epinephelus fuscoguttatus*) showed 11 volatile compounds in fresh fish samples (Table 1) and 19 volatile compounds in steamed fish (Table 2). Compounds in steamed fish samples were higher than fresh fish. The volatile flavor compounds identified were categorized into several groups such as hydrocarbons, aldehydes, alcohols, and ketones.

Aldehyde, alcohol, ketone, acid and hydrocarbon compounds were the main volatile compounds in fishery products (Liu et al. 2009). Fresh groupers had fewer volatile compounds than Steamed Grouper. According to Pratama et al. (2013) it generally could be said that steamed samples would have more volatile amounts than fresh samples. A process involving heat such as steaming was one of the factors affecting the identified volatile compound.

Table 1. Volatile compounds in fresh tiger grouper fish

No.	Group	RT	Compound	Area	Proportion(%)
1	Hydrocarbons	14,48	*Limonene*	33872	5,212
2		18,406	*1H-Indene, 1-methylene-*	21016	3,234
3		22,561	*Undecane*	3227	0,496
4		24,533	*Hexadecane*	34860	5,364
5	Aldehydes	12,053	*Benzaldehyde, 4-ethyl-*	416534	64,103
6		13,695	*2-Hexenal, (E)-*	57133	8,792
7		35,32	*Pentanal*	1076	0,165
8	Alcohols	15,488	*1-Hexanol*	11788	1,814
9	Ketones	6,2359	*2-Heptanone*	3151	0,484
10		13,417	*3-Heptanone, 6-methyl-*	61870	9,521
11		28,183	*2,3-Pentanedione*	5259	0,809

The results of the analysis of volatile flavor compounds, identified compounds consist of several groups. The hydrocarbon group consists of 4 compounds in fresh grouper samples and 10 compounds in steamed grouper fish samples. In the fresh grouper fish samples, the highest proportion compounds is Hexadecane (5.36%) and the steam samples of the largest compound is tetradecyl-oxirane (24.11%). Hexadecane was also detected in raw and re-cooked silver carp (Liu et al. 2009). Tetradecyl-oxirane was also found in surmai fish oil extract (Lanidya et al 2017). It was known that Tetradecyl-oxirane has a faint pleasant aroma (PubChem 2018). The group of hydrocarbon compounds generally had a high threshold aroma, consequently considered to have a smaller effect on the overall aroma of cooking. Guillen and Errecalde (2002) studies of bream fish showed no significant hydrocarbon effect on flavor. Chung et al. (2002) and Linder and Ackman (2002) suggest that groups of alkane compounds generally did not provide significant amounts of odor in foods. Volatile hydrocarbon group compounds might originate from decarboxylation reactions and

carbon chain separation of fatty acids, secondary reactions from carotenoid thermal oxidation and other unsaturated fats (Chung et al 2002; Linder & amp; Ackman 2002; Liu et al 2009).

Table 2. Volatile compounds in steamed tiger grouper fish

No.	Group	RT	Compound	Area	Proportion (%)
1	Hydrocarbons	14,646	*Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-*	105462	6,936
2		14,655	*Limonene*	77751	5,113
3		15,592	*1-Hexanol, 2-ethyl-*	21231	1,396
4		16,482	*Hexadecane*	47381	3,116
5		16,765	*Undecane*	35008	2,302
6		18,471	*Azulene*	47343	3,113
7		20,513	*4-Cyclopentene-1,3-diol, trans-*	11506	0,756
8		24,539	*Tetradecane*	182116	11,978
9		28,536	*2-Penten-4-yn-1-ol, 3-methyl-, (E)-*	4240	0,278
10		30,248	*Oxirane, tetradecyl-*	366700	24,118
11	Aldehydes	12,136	*Benzaldehyde, 4-ethyl-*	35991	2,367
12		4,5069	*Pentanal*	65417	4,302
13		11,794	*2-Nonenal, (E)-*	24501	1,611
14		14,02	*Octanal*	239875	15,777
15		15,139	*Heptanal*	25446	1,673
16		16,277	*Nonanal*	117843	7,750
17	Alcohols	16,482	*1-Nonanol*	55130	3,626
18		16,498	*1-Penten-3-ol*	56816	3,736
19	Ketones	28,179	*2,3-Pentanedione*	625	0,041

Groups of aldehydes in fresh grouper samples detected 3 compounds, in steam samples there are 6 compounds. In the sample of fresh fish 4 ethyl benzaldehyde is the largest proportion of compounds (64.10%). 4-ethyl- Benzaldehyde was known to have the odor like almond / fruity / nutty / creamy bean flavor and is usually found in crayfish wastes (Tanchotikul and Hsieh 1989). Benzaldehyde is the most important contributor to the aroma of fish sauce and has an effect on preserving (Toth & amp; Potthast 1984). Benzaldehyde probably comes from the degradation of Benzaldehyde amino acids also known as an important flavor in the flesh of freshwater lobster, turbot fish, salmon and fish feed. Benzaldehyde and its derivatives can also be adsorbed in fish meat through their environment because these molecules are lipophilic (Josephson et al. 1991). While the sample of steamed fish octanal compounds were the largest proportion (15.77%). The Octanal has an aroma like fatty, soap, lemon (Varlet et al 2007). Aldehydes have a major effect on many other taste substances, even when present in small amounts (Spurvey et al., 1998). Detectable aldehyde group compounds may be derived from the oxidation of the double carbon bonds of unsaturated fatty acids or saturated fatty acids (Cha et al. 1992; Guillen & Errecalde 2002; Sakakibara et al., 1988). The majority of aldehydes can be considered as fat oxidation products (Chung et al 1993).

Group of alcohols in fresh grouper samples were detected with 1 compound, whereas in steam samples there were 2 compounds. In the sample of fresh fish, compound 1-Hexanol is a compound with proportion (1,81%), whereas in sample of steamed fish compound 1-Penten-3-ol is compound with biggest proportion (3,74%). 1-penten-3-ol is a compound that contributes to rancidity (Jonsdottir et al. 2008). According to Girard and Durance (2000) group of alcohols are generally formed by decomposition and hydro peroxides secondary to fatty acids. Alcohol is not considered an important contributor to fat-flavored flavor due to its relatively high odor threshold. Most alcohols have a rancid smell (Cadwallader et al. 1995).

The group of ketones in fresh fish samples identified 3 compounds, with the largest proportion compound being 3-

Heptanone, 6-methyl- (9.52%). 3-Heptanone, 6- methyl- can contribute aromas such as cream and cheese (Karahadian and Lindsay 1989). In steamed fish detected 1 compound 2,3-Pentanedione (0,041%). Pratama (2011) ketones are largely known to be present in volatile substances and are likely to result from fat oxidation (especially unsaturated fatty acids) during heating, in addition thermal degradation, amino acid degradation and maillard reaction are possible mechanisms for the formation of ketone components. The ketones are quite reactive, the sensory evaluation has shown that ketones possess flavor-forming characters and are involved in the formation of aromatic reaction products with food compounds (Toth & Potthast 1984). The number of these components seems to correlate with the fat content in fish. These components are thought to be responsible for odor resembling the oxidized fish oil (Sakakibara et al., 1990).

Analysis of Proximate

Test results on moisture, ash, protein and fat content of fresh groupers and steamed groupers are presented in Table 3. in the average of three replications and standard deviation.

Table 3. Proximate analysis result in tiger grouper fish (%)

Parameters	Fresh	Steamed
Moisture	77,77±0,03	70,76±0,06
Ash	1,97±0,07	2,04±0,03
Protein	18,21±0,5	24,56±0,18
Fat	0,66±0,02	1,64±0,03

Fresh grouper has 77.7% water content and steamed grouper has 70.76% water content. Samples in the form of steamed water decreased when compared to fresh samples. The differences in the results of these measurements can be influenced by the initial moisture content of the raw materials, the type of each commodity tested and the processing it has undergone (Pratama 2011). Fellows (2000) also states that treatment with hot steam can cause the loss of the water content of the space between the cells so as to increase the density of the food and this is what causes the water content in the sample of commodities which had steamed into measurable lower than the sample in a fresh state.

Steamed groupers have higher ash content than fresh groupers. Steamed grouper has ash content of 2.04% while fresh grouper has ash content of 1.97%. The ash content in this study is more influenced by mineral content than with the processing stage of the raw material. This is due to the absence of the addition of certain mineral salts such as through the process of salting or adding certain additives at all stages of the sample steaming procedure. The content of natural ash in fish is affected by species, age, growth phase, season, time of catch, environment and other internal or external factors (Pratama 2011).

Steamed groupers have higher protein values compared to fresh groupers. Fresh grouper has a protein content of 18.21% while steamed grouper has a protein content of 24.56%. Protein content in each sample studied is influenced by living environment, season, condition and length of storage and processing. Processing such as steaming may give a change in the properties of proteins in steamed materials, although not as much as other heating processes used higher temperatures. The water content that exits during steaming will cause higher levels of measured protein in the proximate test (Pratama 2013). The water content contained in the material will also have a major effect on the measured protein content of the ingredients (Sebranek 2009).

Fresh grouper fish has a fat content of 0.66%, while the steamed grouper fish has a fat content of 1.64% an increase in the value of fat content in the grouper steamed variations in the amount of fat contained in each sample is affected by the living, season, food sources, activity, growth phase of the studied sample (Bligh et al., 1988; Doe, 1958). The treatment process using the principle of heating such as drying, curing including steaming will cause some of the fat melted out of the piece parts of the flesh of fish, but the measurement of fat content will also be influenced by the water content measured (Doe 1998), the higher the water content out of the material then the greater the amount of fat (and other nutrients) measured in the proximate test (Pratama dkk 2013).

Conclusion

Analysis of volatile compounds on fresh tiger grouper (*Epinephelus fuscoguttatus*) successfully detected 11 volatiles with 4-ethyl-benzaldehyde (64.10%) as the highest proportion, while in the sample the steamed grouper detected 19 volatiles with tetradecyl-oxirane (24.11%) as the highest proportion. Groups of volatile compounds detected in all samples can be categorized into groups of hydrocarbons, aldehydes, alcohols and ketones.

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